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SCIENTIFIC DATA REVIEWS
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

January 25, 1996

SUBJECT: 004004. Review of Two Mutagenicity Studies on S-Bioallethrin (Esbiol) for Reregistration

PC Code 004004
DP Barcode D220142
Case No. 818517
MRID Nos. 43696501, 43804401

Tox. Chem. No. 025C
Reregistration Case No. 0437
ID No. 004004

TO: Dana Lateulere, CRM # 72
Reregistration Branch
Special Review
and Reregistration Division (7508W)

FROM: Pamela M. Hurley, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (7509C)

Pamela M Hurley 1/25/96

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (7509C)

Roger Gardner 1/26/96 1/26/96

Background and Request:

AgrEvo has submitted 2 mutagenicity studies (mouse lymphoma forward mutation assay (MRID 43696501) and mouse micronucleus assay (MRID 43804401)) in support of reregistration for S-bioallethrin (esbiol). The Toxicology Branch (TB-I) has been asked to review the studies and determine whether or not they satisfy partial mutagenicity requirements for S-bioallethrin.

Toxicology Branch Response:

TB-I has reviewed the two mutagenicity studies and has determined that they are acceptable for regulatory purposes and that they satisfy partial mutagenicity testing requirements for S-bioallethrin. These studies taken together with the previously submitted bacterial gene mutation study (Ames assay) on S-bioallethrin satisfy all of the mutagenicity testing requirements for S-biolallethrin under the new mutagenicity testing guidelines. The following paragraphs summarize the studies.

In a mammalian gene mutation assay at the thymidine kinase (TK) locus in L5178Y TK+/- mouse lymphoma cells (MRID 43696501), cells cultured in vitro were exposed to esbiol (95.3 %a.i.), both with and without metabolic activation. Without activation, the cells were treated up to levels of cytotoxicity: 7.50, 10.0, 15.0, 20.0, 30.0, 40.0, or 45.0 µg/ml. With activation, the cells were treated with 7.50, 15.0, 20.0, 30.0, 40.0 or 59.9 µg/ml.

Esbiol was tested up to cytotoxic concentrations both with and without metabolic activation. The highest mutant frequency recorded without activation was 67.8 X 10^{-6} units versus 67.6 X 10^{-6} in the vehicle control. The highest mutant frequency with activation was 74.0 X 10^{-6} units versus 51.5 X 10^{-6} in the control. The positive controls induced the appropriate responses. **There was no evidence or a concentration related positive response of induced mutant colonies over background.**

In a CD-1 (ICR) mouse bone marrow micronucleus assay (MRID 43804401), 15/sex/dose were treated by gavage with Esbiol (95.3% a.i.) at doses of 75.0, 150 or 300 mg/kg for males and 65.0, 130 or 260 mg/kg for females. Bone marrow cells were harvested in 5 animals/sex at each of 24, 48 and 72 hours post-treatment. The vehicle (corn oil, 10 ml/kg)) and the positive control (cyclophosphamide (CP), 80 mg/kg) were administered by gavage concurrently with the test article. Bone marrow cells for the vehicle and the positive control were harvested at 24 hours.

There were clinical signs of toxicity during the study. These included death, hyperactivity and tremors. Esbiol was tested at an adequate dose. The positive control induced the appropriate response. **No significant increases in the frequency of micronucleated polychromatic erythrocytes were observed in the bone marrow at any of the harvest times.**

Summary of Data Requirements for S-Bioallethrin

The following list summarizes the current data requirements for S-bioallethrin (esbiol) and whether or not they have been satisfied.

Esbiol

		Required	Satisfied
81-1	Acute Oral Toxicity	Yes	Yes
81-2	Acute Dermal Toxicity	Yes	No
81-3	Acute Inhalation Toxicity	Yes	No
81-4	Primary Eye Irritation	Yes	No[1]
81-5	Primary Dermal Irritation	Yes	No[2]
81-6	Dermal Sensitization	Yes	No
82-1(a)	Subchronic Oral (rodent)	Yes	No
82-1(b)	Subchronic Oral (non-rodent)	Yes	No
82-2	21-Day Dermal	Yes	No
83-1(a)	Chronic Toxicity (rodent)	Yes	Yes[6]
83-1(b)	Chronic Toxicity (nonrodent)	Yes	No[3]
83-2	Oncogenicity (mouse)	Yes	No[4]
83-5	Oncogenicity (rat)	Yes	Yes[6]
83-3(a)	Teratology (first species)	Yes	No
83-3(b)	Teratology (second species)	Yes	No
83-4	Multigeneration Reproduction	Yes	Yes[5]
84-2	Mutagenicity - Gene Mutation Bacterial	Yes	Yes
84-2	Mutagenicity - Gene Mutation Mammalian cells	Yes	Yes
84-2	Mutagenicity - Structural Chromosomal Aberrations	Yes	Yes
85-1	Metabolism	Yes	Possibly[7]

[1]Study may possibly be upgraded if scoring method submitted.
[2]This study had an unusual test design. Needs additional information.
[3]The 1-year study on esbiothrin is supplementary, upgradable to guideline upon submission of data from preliminary study. The chronic dog study on esbiothrin will satisfy this requirement if upgraded.
[4]The oncogenicity study in the mouse was conducted on esbiothrin and is graded supplementary pending submission of preliminary range-finding study. The mice could have tolerated higher dose levels. This study will satisfy requirements for esbiol if upgraded.
[5]This requirement has been satisfied by an acceptable reproduction study conducted on esbiothrin.
[6]This requirement has been satisfied by an acceptable chronic/oncogenicity feeding study conducted on esbiothrin.
[7]Metabolism studies were submitted on bioallethrin to cover all the allethrins. The decision on whether or not the studies can cover all the allethrins needs to be considered by HED (possibly the metabolism committee).

EPA Reviewer: Pamela M. Hurley Pamela M Hurley, Date 1/25/96
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Roger Gardner Roger Gardner, Date 1/26/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in the mouse; OPPTS 870.5395 [§84-2]

DP BARCODE: D220142 SUBMISSION CODE: S495392
P.C. CODE: 004004 TOX. CHEM. NO.: 025C

TEST MATERIAL (PURITY): RUC #805 (95.3%)

SYNONYMS: Esbiol, S-bioallethrin

CITATION: Murli, H. (1995) Mutagenicity test on RUC # 805 in an in vivo mouse micronucleus assay. Corning Hazleton Inc. Vienna, Virginia 22182. CHV Study No. 16624-0-455, RUC Study No. T-94-165, April 7, 1995. MRID 43804401. Unpublished.

SPONSOR: AgrEvo Environmental Health, Montvale, New Jersey

EXECUTIVE SUMMARY:

In a CD-1 (ICR) mouse bone marrow micronucleus assay (MRID 43804401), 15/sex/dose were treated by gavage with Esbiol (95.3% a.i.) at doses of 75.0, 150 or 300 mg/kg for males and 65.0, 130 or 260 mg/kg for females. Bone marrow cells were harvested in 5 animals/sex at each of 24, 48 and 72 hours post-treatment. The vehicle (corn oil, 10 ml/kg)) and the positive control (cyclophosphamide (CP), 80 mg/kg) were administered by gavage concurrently with the test article. Bone marrow cells for the vehicle and the positive control were harvested at 24 hours.

There were clinical signs of toxicity during the study. These included death, hyperactivity and tremors. Esbiol was tested at an adequate dose. The positive control induced the appropriate response. **No significant increases in the frequency of micronucleated polychromatic erythrocytes were observed in the bone marrow at any of the harvest times.**

This study is classified as acceptable. It satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: RUC #805
Description: viscous yellow liquid
Lot/Batch #: Lot No. 2N 0680
Purity: 95.3% a.i.
Stability of compound: The report stated that stability is the responsibility of the Sponsor
CAS #'s: stereoisomeric mixture: 28057-48-9; 28434-00-6

The following figure is a representative structure from the allethrin family.

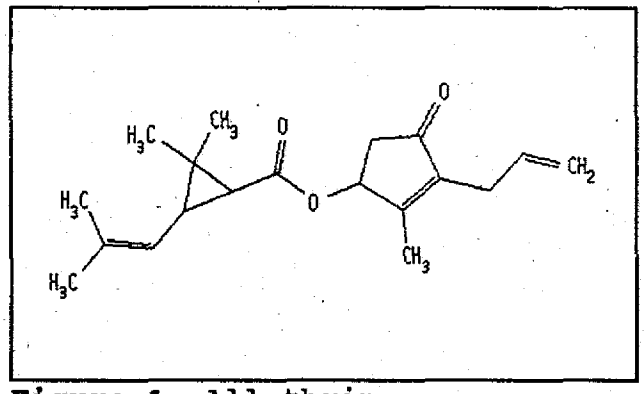

Figure 1 Allethrin

Solvent used: corn oil (Duke's, Lot No. 6202)

2. Control Materials:

Negative/Route of administration: See vehicle

Vehicle/Final volume/Route of administration: Corn oil by gavage in a volume of 10 ml/kg.

Positive/Final dose(s)/Route of administration: Cyclophosphamide (CP) in deionized water by oral gavage at 80 mg/kg.

3. Test compound administration:

Volume of test substance administered: 10 ml/kg.

Route of administration: oral gavage.

Dose levels used: 75.0, 150 or 300 mg/kg for males and 65.0, 130 or 260 mg/kg for females.

4. Test animals:

a. Species mouse Strain CD-1 (ICR) Age not stated
Weight: male 29.9 - 38.5 grams female 22.8 - 29.3 grams
Source: Charles River Laboratories, Portage, Michigan

b. No. animals used per dose: 15 males 15 females; 5/sex for the vehicle and positive controls.

c. Properly maintained? Yes

B. TEST PERFORMANCE

1. Treatment and Sampling Times:

a. Test compound
Dosing: __x__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
__x__ 24 hr __x__ 48 hr __x__ 72 hr (mark all that are appropriate), _____ other (describe):

b. Negative and/or vehicle control
Dosing: __x__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
__x__ 24 hr _____ 48 hr _____ 72 hr (mark all that are appropriate), other (describe):

c. Positive control
Dosing: __x__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
__x__ 24 hr _____ 48 hr _____ 72 hr (mark all that are appropriate), other (describe):

2. Tissues and Cells Examined:

__x__ bone marrow _____ other (list):

No. of polychromatic erythrocytes (PCE) examined per animal: 1000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: _____
Other (if other cell types examined, describe): The frequency of PCEs versus NCEs was determined by scoring the number of PCEs and NCEs observed in the optic fields while scoring the first 1000 erythrocytes.

3. Details of slide preparation: The report stated that "the animals were euthanized with CO_2 and the adhering soft tissue and epiphyses of both tibiae were removed. The marrow was flushed from the bone and transferred to centrifuge tubes containing 3-5 ml bovine serum (one tube for each animal). Following centrifugation to pellet the tissue, the supernatant was removed by aspiration and portions of the pellet were spread on slides and air-dried. The slides were fixed in methanol and stained in May-Grunwald solution followed by Giemsa. The air-dried slides were coverslipped using Depex® mounting medium."

4. Statistical methods The report stated that "the analysis of the data was performed using an Analysis of Variance on the square root arcsine transformation which was performed on the proportion of cells with micronuclei per animal (square root arcsine proportion). Once the Analysis of Variance had been performed, Tukey's Studentized range test (HSD) with adjustment for multiple comparisons was used at each harvest time to determine which dose groups, if any, were significantly different ($p < 0.05$) from the vehicle control. Analyses were performed separately for each harvest time and sex combination, and also at each harvest time for the sexes combined."

5. Evaluation Criteria The report stated that "the criteria for the identification of micronuclei were those of Schmid (1976). Micronuclei were darkly stained and generally round, although almond and ring-shaped micronuclei occasionally occur. Micronuclei had sharp borders and were generally between 1/20 and 1/5 the size of the PCE. The unit of scoring was the micronucleated cell, not the micronucleus; thus the occasional cell with more than one micronucleus was counted as one micronucleated PCE, not two (or more) micronuclei. The staining procedure permitted the differentiation by color of PCEs and NCEs (bluish-grey and red, respectively).

The report also stated that "the criteria for determining a positive response involved a statistically significant dose-related increase in micronucleated PCEs, or the detection of a reproducible and statistically significant positive response for at least one dose level. A test article that induced neither a statistically significant dose response nor a statistically significant and reproducible increase at one dose level was considered negative. In either case, the final decision was based on scientific judgment."

II. REPORTED RESULTS

A. Preliminary toxicity assay: There were 3 dose selection trials. In the first trial, single doses of 500, 1625, 2750, 3875 or 5000 mg/kg body weight were administered to groups of 3/sex/dose by oral gavage in a 10 ml/kg dosing volume. The animals had severe convulsions immediately after dosing. Within 0.75 hours of dosing, all animals had died.

In the second trial, single doses of 40.0, 130, 220, 310 or 400 mg/kg were administered to groups of 3/sex/dose by oral gavage in a 10 ml/kg dosing volume. All animals appeared normal immediately after dosing, except those at the highest dose level, which appeared hyperactive. Within 30 minutes, 2 females from the 400 mg/kg group and 1 female from the 310 mg/kg group were having seizures. All other animals in these 2 dose groups were hyperactive and had tremors. After 1 hour, 2 males and 3 females from the high dose group and 1 female from the 310 mg/kg group were found dead. The surviving male from the high dose group was hyperactive and had severe tremors. All animals in the 310 mg/kg group were hyperactive and had mild tremors. Sixteen hours after dosing, the remaining male from the high dose group and 1 male and 1 female from the 310 mg/kg group had died. **Based on these results, the "maximally tolerated dose (MTD)" for males was calculated from this trial to be approximately 300 mg/kg.** A third trial had to be conducted for females.

In the third trial, single doses of 260 or 280 mg/kg were administered to groups of 3 females/dose by oral gavage in a 10 ml/kg dosing volume. Approximately 1 hour after dosing, 1 female from the 280 mg/kg group had convulsions. After 1.5 hours, the same female was hyperactive and had tremors and seizures. The other two females from the same group also had seizures. All females in the 260 mg/kg group were hyperactive and had tremors. Three and 1/4 hours after dosing 2 females from the high dose group had died. The remaining female was hyperactive and had tremors. The animals in the 260 mg/kg group were all slightly hyperactive. Twenty-one hours after dosing, all remaining females appeared normal. **Based on these results, the "MTD" for females was estimated to be approximately 260 mg/kg.**

The doses for the micronucleus assay were selected based on the results of the 3 trials. These were 75.0, 150 or 300 mg/kg for males and 65.0, 130 or 260 mg/kg for females.

B. Micronucleus assay: None of the animals in the vehicle and positive control groups displayed any clinical signs of toxicity at any time throughout the study. Within 1 hour after dosing 1 female from the 260 mg/kg group had died.

The remainder of the females in this dose group and all males in the 300 mg/kg group were hyperactive and had tremors. Two hours after dosing, 4 males from the 300 mg/kg group and 2 females from the 260 mg/kg group had died. All surviving males from the 300 mg/kg group, all surviving females from the 260 mg/kg group, all males from the 150 mg/kg group and all females from the 130 mg/kg group had tremors. Twenty hours after dosing, 3 males from the 300 mg/kg group and 3 females from the 260 mg/kg group had died. All other animals appeared normal and remained so until study termination.

The test article did not induce any significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle controls in either sex at any of the harvest times. The positive control, CP, induced significant increases in micronucleated PCEs in both sexes at 24 hours. The following table taken from the report summarizes the results.

Micronucleus Data Summary Table							
			% Micronucleated PCEs Mean of 1000 per animal			Ratio PCE:NCE Mean	
Treatment	Dose	Harvest Time (HR)	Males	Females	Total	Males	Females
Vehicle Control Corn oil	10 ml/kg	24	0.04	0.08	0.06	0.41	0.68
Positive Control CP	80 mg/kg	24	2.98*	2.36*	2.67*	0.58	0.76
Test Article	75 mg/kg (male) 65 mg/kg (female)	24 48 72	0.04 0.16 0.00	0.04 0.06 0.06	0.04 0.11 0.03	0.52 0.69 0.65	0.56 0.69 0.95.
	150 mg/kg (male) 130 mg/kg (female)	24 48 72	0.06 0.06 0.10	0.10 0.06 0.04	0.08 0.06 0.07	0.59 0.73 0.57	0.82 0.63 0.88
	300 mg/kg (male) 260 mg/kg (female)	24 48 72	0.14 0.03 0.15	0.02 0.04 0.05	0.08 0.03 0.10	0.67 0.50 0.67	0.65 0.55 1.04

*Statistically significant over controls $p < 0.05$.

III. **REVIEWER'S DISCUSSION/CONCLUSIONS:** This study is acceptable as submitted. The data requirement for an in vivo bone marrow cytogenetics study has been satisfied. There are no major deficiencies in the study. Under the conditions of the study, Esbiol does not induce any significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle controls in either sex at any of the harvest times. The dose levels were adequate for a negative study.

EPA Reviewer: Pamela M. Hurley Pamela M. Hurley, Date 1/24/96
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Roger Gardner Roger Gardner, Date 1/26/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in mouse lymphoma L5178Y cells; OPPTS 870.5300 [§84-2]

DP BARCODE: D220142 SUBMISSION CODE: S495392
P.C. CODE: 004004 TOX. CHEM. NO.: 025C

TEST MATERIAL (PURITY): RUC #805 (95.3%)

SYNONYMS: S-Bioallethrin, Esbiol

CITATION: Cifone, M. (1995) Mutagenicity test on RUC #805 in the L5178Y TK+/- mouse lymphoma forward mutation assay. Corning Hazleton Inc. (CHV) Vienna, Virginia 22182. CHV Study No. 16624-0-431, RUC Study No. T-94-164, April 7, 1995. MRID 43696501. Unpublished.

SPONSOR: Agrevo Environmental Health, Montvale, New Jersey

EXECUTIVE SUMMARY:

In a mammalian gene mutation assay at the thymidine kinase (TK) locus in L5178Y TK+/- mouse lymphoma cells (MRID 43696501), cells cultured in vitro were exposed to esbiol (95.3 %a.i.), both with and without metabolic activation. Without activation, the cells were treated up to levels of cytotoxicity: 7.50, 10.0, 15.0, 20.0, 30.0, 40.0, or 45.0 µg/ml. With activation, the cells were treated with 7.50, 15.0, 20.0, 30.0, 40.0 or 59.9 µg/ml.

Esbiol was tested up to cytotoxic concentrations both with and without metabolic activation. The highest mutant frequency recorded without activation was 67.8 X 10^{-6} units versus 67.6 X 10^{-6} in the vehicle control. The highest mutant frequency with activation was 74.0 X 10^{-6} units versus 51.5 X 10^{-6} in the control. The positive controls induced the appropriate responses. **There was no evidence or a concentration related positive response of induced mutant colonies over background.**

This study is classified as acceptable. It satisfies the requirement for FIFRA Test Guideline 84-2 for in vitro mutagenicity (mammalian cell gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

011780

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: RUC #805
Description: viscous yellow/brown liquid
Lot/Batch #: Lot No. 2N 0680
Purity: 95.3% a.i.
Stability of compound: Not stated
CAS #'s: stereoisomeric mixture: 28057-48-9; 28434-00-6

The following figure is a representative structure from the allethrin family.

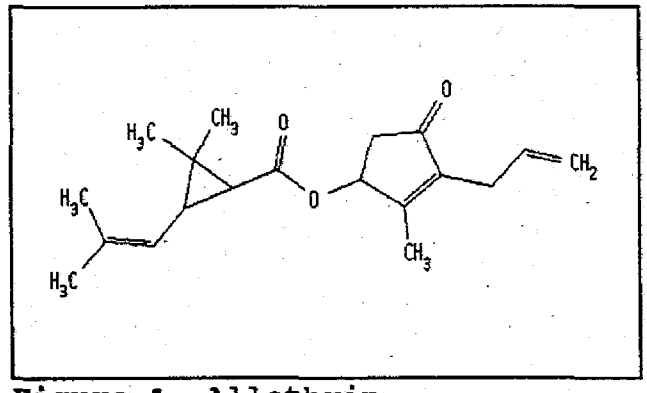

Figure 1 Allethrin

Solvent used: dimethylsulfoxide (DMSO)

2. Control Materials:
Negative: DMSO
Solvent/final concentration: 1%
Positive: Nonactivation: methyl methanesulfonate (MMS) (10 nl/ml and 15 nl/ml)

Activation: 20-methyl cholanthrene (MCA) (2.0 µg/ml and 4.0 µg/ml)

3. Activation: S9 derived from

__x Aroclor 1254	__x induced	__x rat	__x liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

If other, describe below

Describe S9 mix composition (if purchased, give details): The enzymes were prepared commercially (Molecular Toxicology, Inc., Annapolis, MD) where the S9 was tested for sterility and enzyme activity. The final S9 fraction had the following concentrations in culture:

NADP (sodium salt)	3mM
Isocitrate	5mM
S9 homogenate	5µl/ml

The amount of S9 homogenate per culture depended upon the enzyme activity of the individual lots. These were tested using a positive control which requires metabolic activation (20-methyl cholanthrene). The optimum S9 concentration was selected based upon induction of TK-/- mutants in mouse lymphoma cells.

4. Test Cells: mammalian cells in culture
   __x__ mouse lymphoma L5178Y cells
   ___ Chinese hamster ovary (CHO) cells
   ___ V79 cells (Chinese hamster lung fibroblasts)
   ___ other (list):

Properly maintained? Yes
Periodically checked for Mycoplasma contamination? Yes
Periodically checked for karyotype stability? Yes
Periodically "cleansed" against high spontaneous background? Yes

Media: The medium used was "RPMI 1640 (Amacher et al., 1980; Clive et al., 1987) supplemented with Pluronic® F68, L-glutamine, sodium pyruvate, antibiotics, and heat-inactivated horse serum (10% by volume). Treatment medium was Fishers medium with the same media supplements used in the culture medium except that the horse serum concentration was reduced to 5% by volume. Cloning medium consisted of the preceding growth medium with up to 20% horse serum, without Pluronic® F68 and with the addition of BBL purified agar at a final concentration of 0.24 percent to achieve a semisolid state. Selection medium was cloning medium that contained 3 µg/ml of TFT."

5. Locus Examined:
   __x__ thymidine kinase (TK)
   Selection agent: _ bromodeoxyuridine (BrdU)
   _ fluorodeoxyuridine (FdU)
   x trifluorothymidine (TFT)

   ___ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
   Selection agent: _____ 8-azaguanine (8-AG)
   (give concentr. _____ 6-thioguanine (6-TG)

___ Na^+/K^+ ATPase
Selection agent: _____ ouabain
(give concentration)

___ other (locus and/or selection agent; give details):

6. Test compound concentrations used: Without activation, the cells were treated up to levels of cytotoxicity: 7.50, 10.0, 15.0, 20.0, 30.0, 40.0, or 45.0 µg/ml. With activation, the cells were treated with 7.50, 15.0, 20.0, 30.0, 40.0 or 59.9 µg/ml.

B. TEST PERFORMANCE

1. Cell treatment:
a. Cells exposed to test compound, negative/solvent or positive controls for:
4 hours (nonactivated) 4 hours (activated)

b. After washing, cells cultured for 2 days (expression period) before cell selection.

c. After expression, $1x10^6$ cells/dish (3 dishes/group) were cultured for 10-14 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 10-14 days without selective agent to determine cloning efficiency.

2. Statistical Methods: Statistical methods were not discussed.

3. Evaluation Criteria: The assay acceptance criteria were listed as follows:

- The average absolute cloning efficiency of the vehicle controls should be between 60 and 130%. Assays with cloning efficiencies for the vehicle controls in the range of 50-60% are conditionally acceptable and dependent upon the scientific judgement of the study director.

- The minimum value for the suspension growth of the average vehicle controls for 2 days is 8.0 fold increase from the original cell numbers.

- The background mutant frequency is calculated separeately for concurrent activation and nonactivation assays, even if the same population of cells was used for each assay. For both conditions, the normal range of background frequencies for assay performed with different cell stocks is 30 $\bar{x}$ 10^{-6} to 120 X 10^{-6}.

Assays with backgrounds outside this range are not necessarily invalid but will be used with caution.

- A positive control is included with each assay. An assay is acceptable in the absence of a positive control only if the test article clearly shows mutagenic activity.

- For test articles with little or no mutagenic activity, an assay includes applied concentrations that reduce the relative growth to 10-20% of the average vehicle controls or reach the maximum applied concentration given in the evaluation criteria. This requirement is waived if the concentration of the highest assayed dose is at least 75% of an excessively toxic dose level or if the highest assayed dose is at least twice the solubility limit of the test material in culture medium.

- An experimental mutant frequency will be considered acceptable for evaluation only if the relative cloning efficiency is 10% or greater and the total number of viable clones in the selection plating efficiency plates exceeds about 60.

- The mutant frequencies for 5 treated cultures are normally determined in each assay, although a minimum of 3 analyzed cultures is considered necessary under the most favorable conditions to accept a single assay for evaluation of the test material.

- The minimum criterion considered necessary to demonstrate mutagenesis for any given treatment is a mutant frequency that is $\geq$ 2 times the concurrent background mutant frequency.

- A dose-related or toxicity-related increase in mutant frequency should be observed. It is desirable to obtain this relation for at least 3 doses, but this depends upon the concentration steps chosen for the assay and the toxicity at which mutagenic activity appears.

- If the mutant frequency obtained for a single dose at or near the highest testable toxicity is about 2 or more times the minimum criterion, the test material will be considered mutagenic in a single trial. Smaller increases at a single dose near the highest testable toxicity will require confirmation by a repeat assay.

- For some test materials, the correlation between toxicity and applied concentration is poor. Therefore, either applied concentration or toxicity (% relative growth) can be used to establish whether the increase in mutant frequency is related to an increase in effective treatment.

- Treatments that induce less than 10% relative growth are included in the assay, but are not used as primary evidence for mutagenicity as it relates to risk assessment.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay: The report stated that the test material was weakly to noncytotoxic with and without metabolic activation from 1.95 µg/ml to 31.3 µg/ml and treatments at 62.5 µg/ml were highly cytotoxic. Higher conditions were lethal under both activation conditions.

B. Mutagenicity assay: Without activation, 3 assays were conducted, but one assay was terminated because "a good range of cytotoxicities was not available for evaluation." In trial 1, "seven treatments from 7.50 µg/ml to 79.9 µg/ml were initiated and treatments at 59.9 µg/ml and 79.9 µg/ml were terminated because of excessive cytotoxicities." None of the assayed treatments induced a mutation frequency that exceeded a mutant frequency that was twice that of the vehicle controls. Trial 2 showed a shift in cytotoxicity and no cytotoxicity was observed, even as high as 79.9 µg/ml. The trial was terminated prior to cloning. In trial 3, treatments above 45.0 µg/ml were terminated because of excessive cytotoxicities. In the dose levels remaining, there was no indication of mutagenic activity.

With activation, a single assay was conducted. The treatment at 79.9 µg/ml was terminated because of excessive toxicity. Again, there was no indication of mutagenic activity. The average cloning efficiencies for the vehicle controls were 81.8% and 82.0% without activation and 83.9% with activation. The positive controls gave appropriate positive responses for the assay. The following tables taken from the report summarize the results.

S-Bioallethrin MAMMALIAN CELLS IN CULTURE; GENE MUTATION (84-2)

Mutation Assay Without Activation - Trial 1										
Test Condition	Daily Cell Counts (cells/ml.10E5 units)		Suspension Growth[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (10E-6 units)[d]
	1	2								
Nonactivation Controls[e]				AVG Vehicle Control				AVG Vehicle Control		
Vehicle Control	13.2	18.4	27.0		123	517	86.2		100.0	47.6
Vehicle Control	18.3	16.8	34.2		93	417	69.5		100.0	44.6
Vehicle Control	18.4	15.5	31.7	31.0	116	538	89.7	81.8	100.0	43.1
MMS 10 nl/ml	7.5	18.4	15.3		490	225	37.5		22.6	435.6[f]
MMS 15 nl/ml	7.2	12.2	9.8		419	121	20.2		7.8	692.6[f]
Test Compound			Relative to vehicle control (%)				Relative to vehicle control (%)			
7.50 µg/ml	17.6	16.8	106.0		83	494	100.7		106.7	33.6
15.0 µg/ml	17.1	14.7	90.1		97	506	103.1		92.9	38.3
20.0 µg/ml	12.9	21.3	98.5		71	457	93.1		91.7	31.1
30.0 µg/ml	12.3	19.9	87.7		87	419	85.4		74.9	41.5
40.0 µg/ml	11.4	14.6	59.7		100	495	100.9		60.2	40.4

[a]Suspension growth = (day 1 count/3) * (Day 2 count)/(3 or day 1 count if not split back)
[b]Cloning efficiency = total viable colony count/number of cells seeded * 100
[c]Relative growth = (relative suspension growth * relative cloning efficiency)/100
[d]Mutant frequency = (total mutant colonies/total viable colonies) x 2x10E-4. Decimal is moved to express the frequency in units of 10E-6
[e]Vehicle control = 1% DMSO: MMS = methyl methanesulfonate positive control
[f]Mutagenic. Exceeds minimum criterion of 90.2 x 10E-6

S-Bioallethrin MAMMALIAN CELLS IN CULTURE; GENE MUTATION (84-2)

Mutation Assay Without Activation - Trial 3										
Test Condition	Daily Cell Counts (cells/ml.10E5 units)		Suspension Growth[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (10E-6 units)[d]
	1	2								
Nonactivation Controls[e]				AVG Vehicle Control				AVG Vehicle Control		
Vehicle Control	21.1	14.8	34.7		151	514	85.7		100.0	58.8
Vehicle Control	18.3	15.5	31.5		152	529	88.2		100.0	57.5
Vehicle Control	17.2	19.7	37.6	34.6	146**	432	72.0	82.0	100.0	67.6
MMS 10 nl/ml	13.1	11.7	17.0		549	258	43.0		25.8	425.6[f]
MMS 15 nl/ml	8.2	8.7	7.9		407	100	16.7		4.7	814.0[f]
Test Compound			Relative to vehicle control (%)				Relative to vehicle control (%)			
10.0 µg/ml	16.7	17.6	94.4		138**	413	83.9		79.2	66.8
20.0 µg/ml	14.4	18.7	86.5		106	427	86.8		75.1	49.6
30.0 µg/ml	12.7	18.2	74.2		104**	490	99.6		73.9	42.4
40.0 µg/ml	8.5	14.7	40.1		141**	448	91.1		36.5	62.9
45.0 µg/ml	5.6	7.7	13.8		145	428	87.0		12.0	67.8

[a]Suspension growth = (day 1 count/3) * (Day 2 count)/(3 or day 1 count if not split back)
[b]Cloning efficiency = total viable colony count/number of cells seeded * 100
[c]Relative growth = (relative suspension growth * relative cloning efficiency)/100
[d]Mutant frequency = (total mutant colonies/total viable colonies) x 2x10E-4. Decimal is moved to express the frequency in units of 10E-6
[e]Vehicle control = 1% DMSO: MMS = methyl methanesulfonate positive control
[f]Mutagenic. Exceeds minimum criterion of 122.6 x 10E-6
**One plate contaminated: value determined by weight proportion

S-Bioallethrin MAMMALIAN CELLS IN CULTURE; GENE MUTATION (84-2)

Mutation Assay With Activation - Trial 1										
Test Condition	Daily Cell Counts (cells/ml.10E5 units)		Suspension Growth[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (10E-6 units)[d]
	1	2								
Nonactivation Controls[e]				AVG Vehicle Control				AVG Vehicle Control		
Vehicle Control	17.3	16.9	32.5		125	511	85.2		100.0	48.9
Vehicle Control	15.7	18.6	32.4		113	439	73.2		100.0	51.5
Vehicle Control	14.8	17.5	28.8	31.2	128	559	93.2	83.9	100.0	45.8
MCA 2 µg/ml	10.8	16.2	19.4		703	425	70.8		52.5	330.8[f]
MCA 4 µg/ml	8.2	16.4	14.9		693	392	65.3		37.2	353.6[f]
Test Compound			Relative to vehicle control (%)				Relative to vehicle control (%)			
7.50 µg/ml	9.8	17.1	59.7		161	551[g]	109.5		65.4	58.4
15.0 µg/ml	10.2	13.9	50.5		189	669	132.9		67.1	56.5
20.0 µg/ml	10.2	18.4	66.8		164	443	88.0		58.8	74.0
30.0 µg/ml	9.5	15.3	51.8		160	500	99.3		51.4	64.0
40.0 µg/ml	11.1	14.1	55.7		170	489	97.1		54.1	69.5
59.9 µg/ml	5.7	13.1	26.6		141	427	84.8		22.6	66.0

[a]Suspension growth = (day 1 count/3) * (Day 2 count)/(3 or day 1 count if not split back)
[b]Cloning efficiency = total viable colony count/number of cells seeded * 100
[c]Relative growth = (relative suspension growth * relative cloning efficiency)/100
[d]Mutant frequency = (total mutant colonies/total viable colonies) x 2x10E-4. Decimal is moved to express the frequency in units of 10E-6
[e]Vehicle control = 1% DMSO: MCA = methylcholanthrene positive control
[f]Mutagenic. Exceeds minimum criterion of 97.5 x 10E-6
[g]One plate contaminated: value determined by weight proportion

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III. REVIEWER'S DISCUSSION/CONCLUSIONS:

The study is acceptable. Esbiol does not appear to induce any increases in forward mutations in the TK locus in the L5178Y mouse lymphoma cell line at dose levels up to cytotoxicity. There are no major study deficiencies.

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Chemical:	**S-Bioallethrin**
PC Code:	**004004**
HED File Code	**13000 Tox Reviews**
Memo Date:	**02/01/96**
File ID:	**TX011780**
Accession Number:	**412-01-0073**

HED Records Reference Center
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